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A COMPARATIVE STUDY ON MICROBIAL DIVERSITY IN COMMUNITY VERMICOMPOST, COMMERCIAL VERMICOMPOST, HOUSEHOLD COMPOST

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ABSTRACT: Composting is an eco-friendly biologically driven process wherein micro-organisms break down organic compounds into nutrient-rich fertilizer. Vermicomposting is a non-thermophilic biological oxidation process of composting where certain species of earthworm are used to enhance the process of conversion of organic waste to compost. The study investigates the composition of compost samples derived from commercial vermicompost, vermicompost from Swachagraha Kalika Kendra and house-hold compost. A total of 18 bacterial species and 5 fungal species were observed from the compost. The common bacterial species identified were *E. coli, Staphylococcus spp*, *Bacillus spp, Klebsiella spp*, *Neisseria spp*, *Moraxella spp*, *Pseudomonas spp*, *Brevibacterium spp*, *Streptobacillus spp, Citrobacter spp*, *Proteus spp, Salmonella spp*, *Serratia spp*, Aeromonas spp and Providencia spp. The fungal isolates were identified as *Aspergillus*, *Penicillium*, *Geotrichum*, yeast and *Cladosporium*. Some of them exhibit beneficial traits including nitrogen fixation, phosphate solubilization, potassium solubilization, silicate solubilization, cellulase production and hydrogen cyanide production.

The study showed that the vermicompost collected from Swachagraha Kalika Kendra and home compost had several microorganisms that proves to impart positive impact on the compost, thereby enhancing plant growth. Commercial vermicompost lacked beneficial microorganism.

KEYWORDS: Waste management, composting, vermicompost, bacteria, fungi

INTRODUCTION

Composting is a constructive way of recycling organic waste material like leaves, farm wastes, restaurant wastes, domestic wastes, animal manure, paper products and sewage sludge in an environmentally friendly way, by the action of fungi, bacteria and other organisms, in a warm, moist and aerobic environment, which helps in enriching the soil with nutrients to improve its fertility for healthy plant growth. The microorganisms in the compost use carbon and nitrogen as their energy source, and along with water and oxygen, produce carbondioxide, heat, water[1]. It also doubles as a resourceful way to dispose waste organic material, helping build a circular economy[2,3].Microorganisms like *Trichoderma* spp*, Gliocladium virens, Flavobacterium balustinum, Xanthomonas maltophilia, and Pseudomonas putida* present in compost can act as a biocontrol agent acting as antagonists to certain pathogens, while also improving the overall health of the soil and increasing its nutrient levels[4]. Compost has shown to suppress a variety of soil-born plant diseases like *Rhizoctonia* root rot, *Fusarium* wilt, Corky root rot [4,5]. The microbiota of the compost

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regulates the progression of composting, and the quality of the product. The actions of the microbiota depend on the physical parameters of the compost and the nutrients available[2].

Vermicomposting is a non-thermophilic biological oxidation process to convert organic material to vermicompost, and uses microorganism and earthworms that are active between 10°C to 32°C [6,7] . It is a faster way than regular composting to obtain a product that is beneficial for plant growth. The vermicompost formed has high porosity, drainage, water holding capacity, aeration, low C: N ratios, an increased content of magnesium, ammonium and nitrates, and has a comparatively uniform size when compared to regular compost, which has a more heterogeneous composition [7,8]. Additionally, vermicompost contains significant levels of nutrients present in plant-available forms, such as phosphorus, soluble potassium, exchangeable calcium and nitrates. The treatment of soils with vermicompost helps in fighting plant pathogens and promoting the growth of beneficial rhizospheric microorganisms [9,10].

The aim of the study was to examine the microbial diversity—specifically the bacterial and fungal communities—present in three different compost samples: commercial vermicompost, community vermicompost obtained from Swachagraha Kalika Kendra in Bangalore, and household compost

MATERIALS AND METHODS

Sample collection:

Compost samples were collected from a local community Solid Waste Management and Learning Centre (Swachagraha Kalika Kendra), aerobic compost from a local residents and a commercially- available vermicompost. These samples were collected in sterile zip-lock packets to avoid invasion of outside air microflora.

METHODS

Each of the compost sample was diluted in 10ml of distilled water which gives 10^{-1} dilution. Appropriate dilutions were made and inoculated on nutrient agar (HiMedia Laboratories Pvt Ltd), to observe bacterial species and rose bengal agar (HiMedia Laboratories Pvt Ltd), to observe fungal colonies. The fungal colonies were identified by observing their morphological features using lactophenol cotton blue staining protocol. The total bacterial count was estimated using Standard Plate Count (SPC) technique.

Characterization and identification of bacteria

The bacterial strains isolated were characterized on the basis of their morphological, microscopic and biochemical properties. The colony morphologies were then studied on naked eye. The parameters taken were colour of the colony, solubility on water, opacity, elevation, texture and consistency. Each bacterial isolates were stained to observe Gram character[11]. Further, the bacterial strains were identified by performing biochemical test that included IMViC tests (Indole, Methyl red, Voge's Proskauer, Citrate), Oxidase test and TSI test (Triple sugar iron) [12] .The reagents used for the biotyping were obtained from Hi-Media. The fungus species weres identified by lacto phenol cotton blue staining method with the key characters[13].
The results were tabulated after comparison, and the genus of the organisms was identified based on the observed positive and negative results with reference to Bergey's manual[14].

RESULTS AND DISCUSSION

Different type of bacterial colonies appeared in the agar culture plates. The colonies were found with different colour, size, texture, elevation, edge, and shape. The isolated bacterial strains were characterized by colony morphology study, microscopy study and biochemical characterization. Differences in protein and fat metabolism, carbohydrate metabolism, enzyme production, and the ability to utilize various compounds were the key factors used in the identification of bacteria. Biochemical tests exploit these metabolic and enzymatic differences to distinguish between bacterial species. In contrast, the identification of higher

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plants and animals is primarily based on morphological characteristics, including both external and internal structural differences. There are many bacterial species sharing similarities in size, shape, etc. and can only be differentiated with detection of certain properties that can be recognized using biochemical tests. All species of bacteria possess a unique set of metabolic activates which are controlled by bacterial enzymes. These bacterial enzymes determine the type of test suitable in recognition of structural differences and metabolic activities. The vermicompost collected displayed a wide spectrum of bacterial and fungal species (Fig. 1-6). The colony characteristics of bacterial and fungal colonies are summarized in Table 1, 2, 3,4,5 and 6. All bacterial colonies isolated from commercial vermicompost were Gram-negative (Fig. 2A), whereas samples of vermicompost collected from Swachagraha Kalika Kendra and household compost exhibited a mixed population of both Gram-positive and Gram-negative bacteria (Fig.4A,6B).

Biochemical characterization was carried out using , indole test, methyl red test, Voges Proskauer test, citrate utilization test and catalase test. The biochemical characteristics of bacterial isolates are summarized in Table 7,8 and 9.

Indole test was done for screening the ability of an organism to degrade the amino acid tryptophan and produce indole. When Kovac's reagent is added into the 24 hours culture broth a cherry red ring is formed which indicates a positive result[15],[16]. Therefore, the organism had the ability to degrade tryptophan and produce indole. Most of the microorganisms were found to be negative for Indole test.

Methyl Red test was used to determine the organism's ability to ferment glucose and produce an acid. Methyl red (pH indicator) solution changes the colour of the 24-hour culture broth into red colour if the solution turned acidic due to the production of acid [15,16].Majority of the microorganisms were found to be positive.

Voges Proskauer test was used to determine the organism's ability to produce acetyl methyl carbinol from the glucose fermentation. On the addition of Barritt reagent A and Barritt reagent B, the appearance of pink-red colour in the culture broth indicates positive result[15.16].Most of the organisms were found to be negative for Voges Proskauer test.

Citrate test is used to screen a bacterial isolate for the ability to utilize citrate as its carbon and energy source. The subsequent increase in the pH of the medium is demonstrated by the colour change of a pH indicator from green to blue [15,16].Citrate test was found to be positive for majority of organisms.

Catalase test facilitates the detection of the enzyme catalase in the bacteria. On the addition of H_2O_2 solution, the formation of effervescence indicates positive result [15,16]. Majority of organisms showed positive results for catalase test.

In commercial vermicompost, bacterial species identified for the genus *Providencia spp*, *Citrobacter spp*, *Proteus spp* were found (Table: 7). *Citrobacter spp* showed irregular, entire, flat, opaque, mucoid, and cream colonies and upon performing Gram staining, it was observed to be Gram negative in nature. Colonies on the MRBA plates were studied for their characteristics (Table: 4) and microscopic observation, upon staining, showed spores of *Aspergillus* (Fig: 2B) and yeast cells (Fig: 2C).

The vermicompost collected from Swachagraha Kalika Kendra displayed a wide spectrum of bacterial and fungal species (Table: 2, 5). Among the bacterial species, the most abundantly observed were *Staphylococcus spp, Klebsiella spp, Streptobacillus spp*. Each of them showed differing colony characteristics on nutrient agar plates (Table 8, Fig: 3). *Staphylococcus spp* were identified based on its irregular, flat, matte, opaque and colourless colonies, Gram positive cocci, of uniform size, in clusters and biochemical assays. *Pseudomonas spp* showed punctiform, entire, raised, smooth translucent, and colourless colonies and were observed as Gram negative rods.

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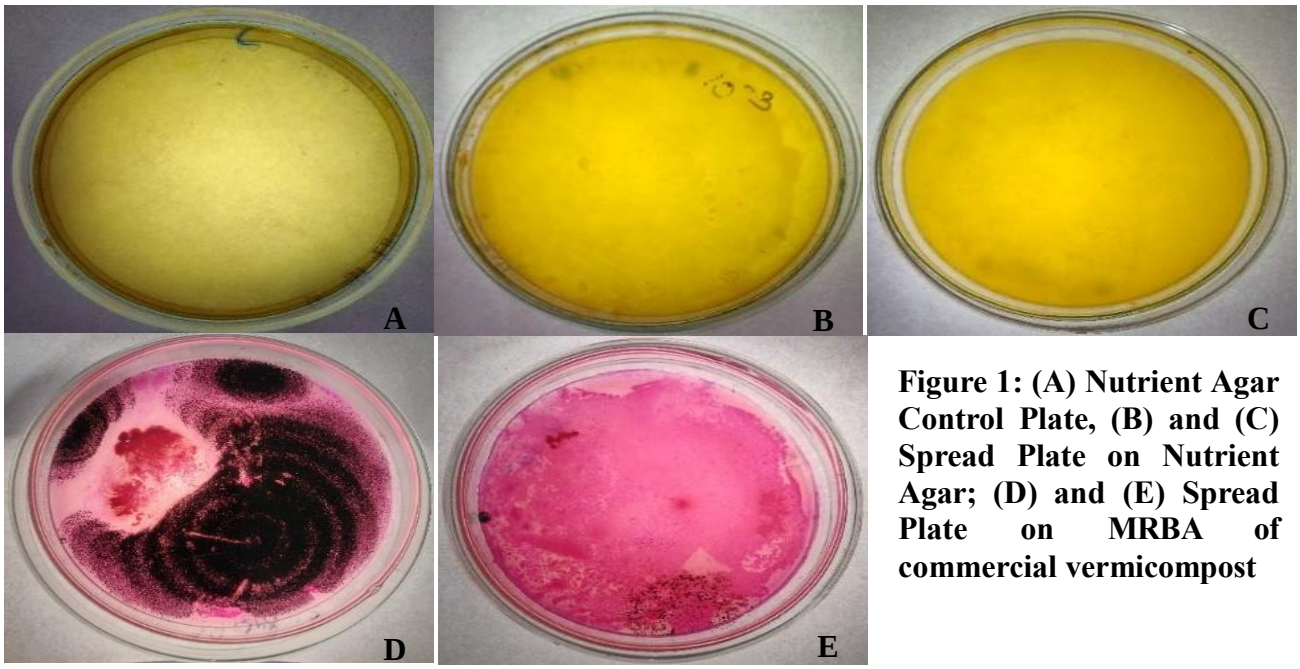

Figure 1: (A) Nutrient Agar Control Plate, (B) and (C) Spread Plate on Nutrient Agar; (D) and (E) Spread Plate on MRBA of commercial vermicompost

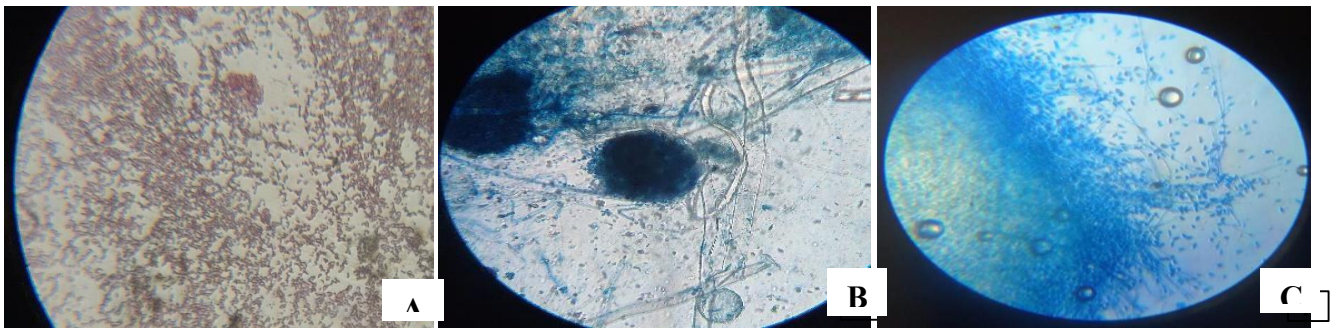

Figure 2 : (A) Gram Staining; (B) and (C) Fungal Staining of commercial vermicompost

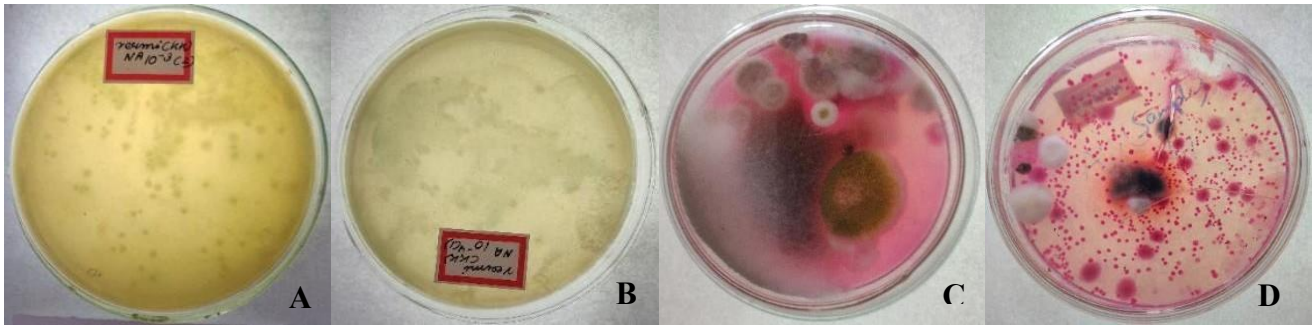

Figure 3: (A) and (B) Spread Plate on Nutrient Agar; (C) and (D) Spread Plate on MRBA of vermicompost from Swacha Kalika Kendra

Some of the other bacterial species observed were *Moraxella, Flavobacterium*. The fungal species were studied for their morphology on MRBA plates followed by observing their spores and were identified to be *Penicillium, Geotrichum, Cladosporium, Aspergillus* and yeast.

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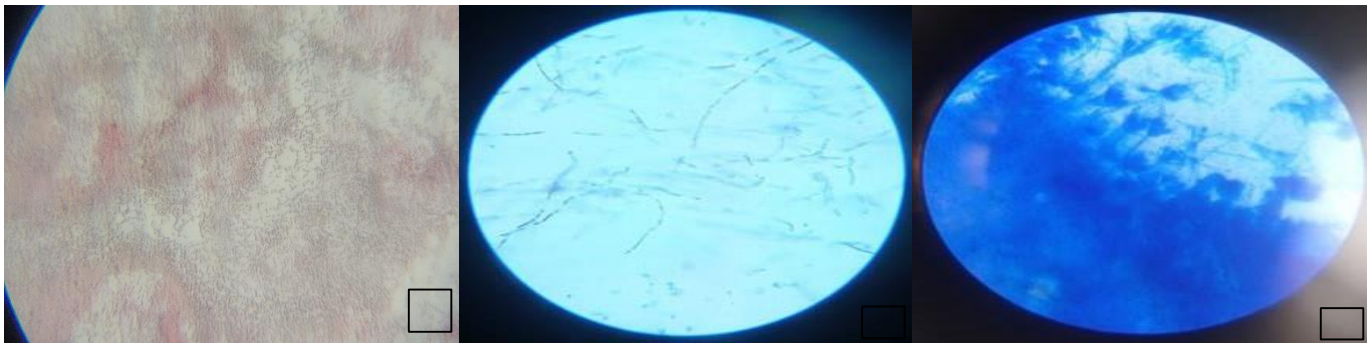

Figure 4: (A) Gram Staining; (B) and (C) Fungal Staining vermicompost from Kalika Kendra

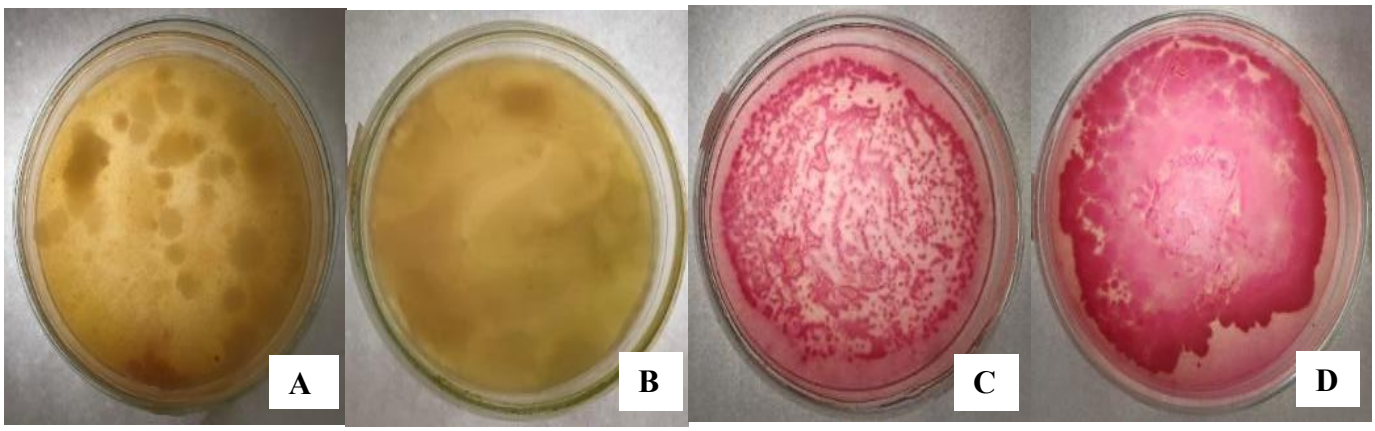

Figure 5: (A) and (B) Spread Plate on Nutrient Agar; (C) and (D) Spread Plate on MRBA of Household Compost

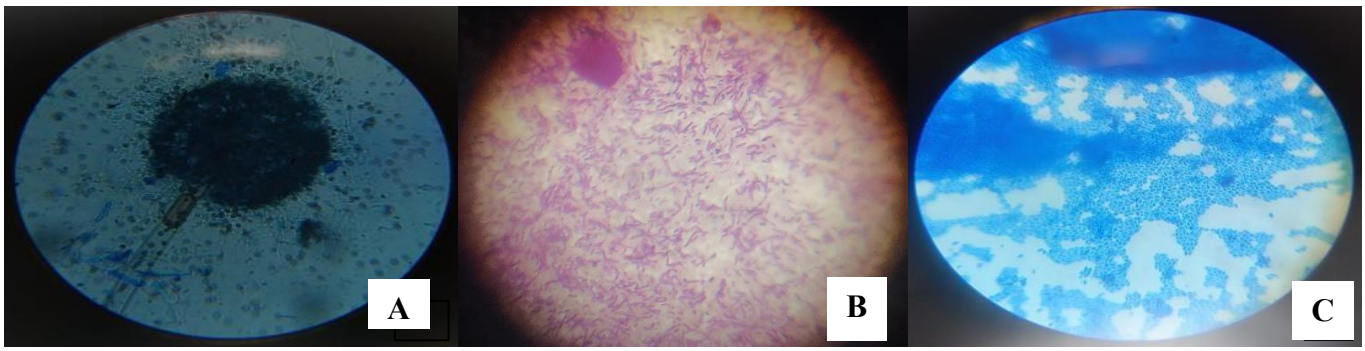

Figure 6 : (A) Fungal Staining; (B) Gram Staining; (C) Fungal Staining of Household Compost

Geotrichum (Fig: 3B) had round, dry, off-whitecolonies and *Penicillium* (Fig: 3C) had filamentous, powdery, yellow-green colonies

Household compost collected from local residents showed the presence of *Klebsiella spp, Escherichia coli, Moraxella spp, Neisseria spp,*

Pseudomonas spp, Salmonella spp, Brevibacterium spp, Bacillus spp and *Stenotrophomonas* (Table: 9, Fig: 9B).

Klebsiella spp and *Escherichia coli* were seen as large, irregular, undulate, umbonate, mucoid, translucent, and white colonies on nutrient agar. The fungal plates showed black, powdery, and irregular colonies (Table: 6) which when microscopically observed, after standard staining protocol, was discerned to be *Aspergillus* (Fig: 9A). Irregular mucoidal and colourless colonies of yeast were also observed (Fig: 9C).

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A comparative analysis of the three samples revealed the presence of several bacterial species common to both the community compost from Swachagraha Kalika Kendra and the household compost. These were *Bacillus spp, Klebsiella spp* and *Pseudomonas spp*. These microorganisms have the potential to improve plant growth under abiotic stress conditions by promoting the production of low-molecular-weight osmolytes, such as glycine betaine, proline, and other amino acids, mineral phosphate solubilization, nitrogen fixation, organic acids, and producing key enzymes such as ACC-deaminase, chitinase and glucanase multi-metal tolerance mechanisms in bacteria[17].

Bacillus spp was the major organism seen in house hold compost. They are gram positive rod. Some important species include *Bacillus subtilis, Bacillus licheniformis, Bacillus circulans*. These bacterial species improve compost maturity, promote lignocellulose degradation, and improve soil fertility[18].

Abundantly observed organisms such as *Bacillus spp and Pseudomonas spp* are known to promote plant growth by suppressing pathogenic micro-organisms, synthesizing growth-stimulating plant hormones and promoting increased plant disease resistance[19,20].

Vermicompost collected from Swachagraha Kalika Kendra commonly harbored potent fungal organisms, including species of *Aspergillus spp, Penicillium spp, Geotrichum spp, Cladosporium spp* as well as various yeasts imparts positive impact on plants growth and are source of phytohormones, enzymes, and amino acids, which significantly promote plant growth and biomass production even under conditions of water stress. *Aspergillus spp* are saprophytic fungus and secretes large amount of various degrading enzymes involved in the decomposition activity and yeast is responsible for decomposition of many complex plant polymers in compost, they break down tough debris enabling bacteria to continue the decomposition process and can produce physiologically active quantities of auxins, which have pronounced effects on plant growth and development. In addition to phytohormone production, microbes can also enhance nutrient uptake through several mechanisms[21].

Some pathogenic organisms were found in commercially-available vermicompost such as *Salmonella spp, Serratia spp and Klebsiella spp*. *Salmonella spp* are able to attach and adhere to plant surfaces before actively infecting the interior of different plants, leading to colonization of plant organs[22] and *Klebsiella pneumoniae* was also found to affect the respiratory mechanism of plant root system in Solanaceous plants[23]. The majorly found bacteria was *Citrobacter spp*. They are opportunistic pathogen and causes effect on human health like urinary tract infections, blood stream infections, intra-abdominal sepsis, brain abscesses, and pneumonia and other neonatal infection. *Citrobacter spp* causes infection of rhizome and leaves (*Zingiber officinale* Rosc). The pathogen infects the rhizomes and leaves. The symptoms include leaf chlorosis and gradual wilting, and softening of the rhizome. In serious cases, the interior of the rhizome is completely eroded, with grey-white juice overflowing the epidermis and a foul smell.

The study concluded that vermicompost obtained from the community composting center, Swachagraha Kalika Kendra, exhibited rich microbial diversity, which has the potential to enhance soil nutrient content and promote plant growth.

CONCLUSION

Composting is a controlled, aerobic process that converts organic materials into a nutrient-rich soil amendment or mulch through natural decomposition. The product, compost, is a dark, crumbly, earthy-smelling materials that improves soil ability to hold nutrients and delivers much needed nutrients required to promotes plant growth and improve soil fertility. The composting process involves various microorganisms including wide range of bacteria, fungi and actinomycetes.

The study was conducted to identify microorganisms from compost collected from

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three sources - vermicompost collected from Swachagraha Kalika Kendra, Bangalore, household compost and a commercially-available compost. The study showed the presence of beneficial microorganisms like *Staphylococcus spp, Pseudomonas spp, Bacillus spp* in vermicompost collected from Swachagraha Kalika Kendra and house hold compost. These beneficial microorganisms promote plant growth by suppressing pathogenic microorganisms, synthesizing growth hormones, and promoting increased plant resistance. The commercial vermicompost showed the presence of pathogenic microorganisms such as *Citrobacter spp*, *Providencia spp* but compost collected from Swachagraha Kalika Kendra and household compost had beneficial microorganisms such as *Bacillus spp, Staphylococcus spp, Pseudomonas spp.*

The benefits of compost include providing nutrients to crops as fertilizers, increasing the humus and humic acid in soil, and suppressing the growth of pathogenic strains due to inhibitory effects of the beneficial microorganisms. It can be concluded, that the compost collected from Swachagraha Kalika Kendra and house hold compost had beneficial microorganisms that promote the soil fertility and growth of plants and serve as an effective way of waste management.

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Table 1: **Colony characteristics for Commercial Vermicompost**

Sl. No.	Size	Shape	Margin	Elevation	Texture	Opacity	Pigmentation	Density	Gram Character
1	Medium	Irregular	Undulate	Flat	Smooth	Opaque	Colourless	1	Gram negative rods
2	Large	Irregular	Entire	Flat	Smooth	Translucent	Colourless	1	Gram negative rods
3	Small	Irregular	Lobate	Raised	Mucoid	Opaque	Cream	1	Gram positive rods
4	Punctiform	Circular	Entire	Flat	Mucoid	Opaque	Cream	1	Gram negative rods
5	Large	Irregular	Entire	Flat	Smooth	Translucent	Colourless	1	Gram negative rods
6	Small	Circular	Entire	Flat	Mucoid	Opaque	Cream	1	Gram negative rods
7	Large	Circular	Entire	Flat	Dry	Translucent	Colourless	1	Gram negative, cocci in clusters
8	Large	Circular	Entire	Flat	Smooth	Translucent	Colourless	1	Gram negative rods

- The experiments were done in triplicates

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Table 2: **Colony characteristics of Vermicompost from Kalika Kendra**

Sl. No.	Size	Shape	Margin	Elevation	Texture	Opacity	Pigmentation	Density	Gram Character
1	Large	Irregular	Undulate	Flat	Matte	Opaque	Colourless	1	Gram positive cocci
2	Medium	Circular	Entire	Flat	Smooth	Translucent	Colourless	TNTC	Gram negative rods + Gram negative cocci
3	Large	Irregular	Undulate	Flat	Smooth	Opaque	Colourless	4	Gram negative rods in chains
4	Medium	Circular	Entire	Raised	Mucoid	Opaque	Colourless	1	Gram negative cocci
5	Punctiform	Punctiform	Entire	Raised	Smooth	Translucent	Colourless	TNTC	Gram negative rods
6	Medium	Circular	Entire	Flat	Smooth	Translucent	Colourless	TNTC	Gram negative rods
7	Large	Irregular	Serrate	Flat	Smooth	Translucent	Colourless	1	Gram positive cocci
8	Large	Irregular	Undulate	Flat	Smooth	Opaque	Colourless	1	Gram negative cocci in clusters
9	Large	Irregular	Undulate	Flat	Matte	Opaque	Colourless	1	Gram negative rods
10	Medium	Irregular	Undulate	Flat	Smooth	Opaque	Colourless	TNTC	Gram positive rods
11	Small	Circular	Entire	Flat	Smooth	Opaque	Colourless	TNTC	Gram negative rods
12	Large	Irregular	Undulate	Flat	Matte	Translucent	Colourless	1	Gram negative rods
13	Medium	Circular	Entire	Flat	Smooth	Transparent	Green tinge	3	Gram negative rods in chains

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Table 3: **Colony Characteristics of Household Compost**

Sl. No.	Size	Shape	Margin	Elevation	Texture	Opacity	Pigmentation	Density	Gram Character
1	Large	Irregular	Lobate	Umbonate	Mucoid	Translucent	Cream	1	Gram negative rods
2	Small	Circular	Entire	Flat	Mucoid	Opaque	White	3	Gram negative rods + Gram negative cocci
3	Small	Irregular	Undulate	Raised	Mucoid	Opaque	White	2	Gram positive rods in chains
4	Punctiform	Punctiform	Entire	Raised	Mucoid	Translucent	White	TNTC	Gram positive rods in chains
5	Large	Irregular	Entire	Flat	Powdery	Translucent	Green tinge	1	Gram negative short rods + Gram negative rods in chains
6	Large	Irregular	Undulate	Umbonate	Mucoid	Translucent	White	1	Gram negative rods + Gram negative cocci

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Table 4: **Fungal colony characteristics observation for Commercial Vermicompost**

Shape	Texture	Colour	Organism Identified
Irregular	Powdery	Black	*Aspergillus*
Circular	Mucoidal	Colourless	*Yeast*
Irregular	Mucoidal	Colourless	*Yeast*
Irregular	Powdery	Black	*Aspergillus*
Circular	Mucoidal	Colourless	*Yeast*

Table 5: **Fungal colony characteristics of Vermicompost from Kalika Kendra**

Shape	Texture	Colour	Organism Identified
Filamentous	Powdery	Yellow/Green	*Penicillium*
Round	Dry	Off-white	*Geotrichum*
Filamentous	Velvety	Olive green	*Cladosporium*
Irregular	Powdery	Black	*Aspergillus*
Punctiform	Smooth	Pink	*Yeast*
Round	Dry	Off-white	*Geotrichum*
Round	Dry	Off-white	*Geotrichum*
Punctiform	Smooth	Pink	*Yeast*
Punctiform	Smooth	Pink	*Yeast*
Punctiform	Smooth	Pink	*Yeast*
Round	Dry	Off-white	*Geotrichum*

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Table 6: **Fungal colony characteristics for Household Compost**

Shape	Texture	Colour	Organism Identified
Punctiform	Smooth	Pink	*Yeast*
Irregular	Powdery	Black	*Aspergillus*
Irregular	Powdery	Black	*Aspergillus*
Punctiform	Smooth	Pink	*Yeast*
Punctiform	Smooth	Pink	*Yeast*
Punctiform	Smooth	Pink	*Yeast*

Table 7: Biochemical tests for Commercial Vermicompost

Sl. No.	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Catalase Test	Suspected Organism
1	Positive	Positive	Negative	Positive	Positive	*Aeromonas spp*
2	Positive	Positive	Negative	Positive	Positive	*Citrobacter spp*
3	Positive	Positive	Negative	Positive	Positive	*Providencia spp*
4	Positive	Positive	Negative	Positive	Positive	*Citrobacter spp, Proteus spp*
5	Positive	Positive	Negative	Positive	Positive	*Citrobacter spp*
6	Positive	Positive	Negative	Positive	Positive	*Citrobacter spp, Proteus spp*
7	Positive	Positive	Negative	Positive	Positive	*Diplococci spp*
8	Positive	Positive	Negative	Positive	Positive	*Salmonella spp, Serratia spp*

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Table 8: Biochemical tests for Vermicompost from Kalika Kendra

Sl. No.	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Catalase Test	Suspected Organism
1	Negative	Negative	Positive	Negative	Positive	*Staphylococcus spp*
2	Negative	Negative	Positive	Negative	Positive	*Escherichia coli*
3	Negative	Negative	Positive	Negative	Positive	*Streptobacillus spp*
4	Negative	Negative	Positive	Negative	Positive	*Moraxella spp*
5	Negative	Negative	Positive	Negative	Positive	*Pseudomonas spp*
6	Negative	Negative	Positive	Negative	Positive	*Klebsiella spp*
7	Negative	Positive	Negative	Negative	Positive	*Staphylococcus spp*
8	Negative	Negative	Positive	Negative	Positive	*Moraxella spp*
9	Negative	Negative	Positive	Negative	Positive	*Pseudomonas spp*
10	Negative	Negative	Positive	Negative	Positive	*Flavobacterium spp*
11	Negative	Negative	Positive	Negative	Positive	*Klebsiella spp*
12	Negative	Negative	Positive	Negative	Positive	*Klebsiella spp*
13	Negative	Negative	Positive	Negative	Positive	*Streptobacillus spp*

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Table 9: Biochemical tests for Compost from Household

Sl. no.	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Catalase Test	Suspected organism
1	Negative	Positive	Negative	Positive	Negative	*Klebsiella spp*
2	Negative	Positive	Negative	Positive	Negative	*Escherichia coli, Klebsiella spp, Neisseria spp, Moraxella spp*
3	Negative	Positive	Negative	Positive	Positive	*Brevibacterium spp, Bacillus coagulans, Bacillus licheniformis*
4	Positive	Positive	Negative	Positive	Negative	*Bacillus circulans, Bacillus pumilus, Bacillus sphericus*
5	Negative	Positive	Negative	Positive	Positive	*Pseudomonas spp, Stenotrophomonas spp*
6	Negative	Negative	Negative	Positive	Negative	*Klebsiella spp, Salmonella spp, Escherichia spp*